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ON THE SO-CALLED PHYSICAL CHEMISTRY OF HEMOLYTIC SERUM.*†

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A FEW years ago, there were assigned to certain serum phenomena a number of physico-chemical laws. It is immaterial for our present purpose what these laws are. Their importance, however, to the future of experimental medicine is evident from the fact that, if true, they not only furnish the first accurately demonstrated facts regarding the molecular composition of certain immunity substances, but they give medical science an instrument by means of which the exact chemical composition of these most important substances might eventually be worked out.

What is apparently the simplest of the phenomena to which these laws were applied, is the phenomenon of the absorption of hemolytic amboceptor by blood corpuscles. It is apparently a simple thing to expose washed corpuscles to accurately measured quantities of heated hemolytic serum, to allow them to stand in contact for definite periods of time, and then to centrifuge the serum free from corpuscles and determine by analysis the amount of unabsorbed amboceptor remaining in it.

In attempting to verify the proposed law,¹ however, numerous experimental difficulties were encountered. Contradictory and even paradoxical results were obtained.² These findings were explained by the subsequent discovery that heated hemolytic serum is so altered by contact with corpuscles as to render it unanalyzable by direct quantitative methods.³

If one is given an HCl-solution of unknown strength, and is asked to determine its strength in terms of a standard HCl-solution, it is a

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¹ For technique, material, etc., see *Jour. Infect. Dis.*, 1905, 2, p. 461.

² *Jour. Infect. Dis.*, 1905, 2, p. 485; *Centralbl. f. Bakt.*, 40, p. 383.

³ *Jour. Infect. Dis.*, 1905, 2, p. 493; *Centralbl. f. Bakt.*, 40, p. 386; *Jour. Biol. Chem.*, 1905, 1, p. 213.

very simple matter to titrate samples of the unknown solution, and calculate its strength as a certain percentage of that of the standard solution.

Suppose, however, that a 4 c.c. sample is taken, and that it is found by titration to contain apparently 20 per cent as much HCl as an equal volume of the standard solution. Suppose, further, that a duplicate titration with a 3 c.c. sample gives apparently 40 per cent; a third titration, with 2 c.c., 60 per cent; a fourth, with 1 c.c., 75 per cent; and a fifth, with $\frac{1}{2}$ c.c., 90 per cent. We would say that the unknown solution was quantitatively unanalyzable, because duplicate titrations do not agree.

This hypothetical result is difficult to imagine, when one is dealing with simple, inorganic substances. It is exactly the kind of result, however, that is obtained when one attempts to determine the residual amboceptor in exposed heated hemolytic serum. Duplicate analyses do not agree, and differ as widely as the results indicated in the hypothetical problem above. The exposed serum is quantitatively unanalyzable.

What is the cause of this phenomenon?

Heated hemolytic serum contains, not only amboceptor, but other substances as well. These are substances that are either originally present in the serum, or that are formed during the heating necessary to destroy the complement. These substances, for convenience, have been spoken of as the "third serum component."¹

Investigation shows that the third component may at times be antilytic, or hemolysis-inhibiting, in its action, and at other times auxilytic, or hemolysis-increasing. Also, that the third component differs in different animals and under different experimental conditions, and that it apparently consists of a mixture of a number of quite distinct substances, each with its specific effect on hemolysis.

It is found that the third component is altered by exposure to corpuscles. A third component that originally exercises a stimulating action on hemolysis has that action decreased by such exposure, or even replaced by an inhibiting action. And a third component that originally exercises an inhibiting action has its inhibiting action increased.

¹ *Jour. Infect. Dis.*, 1906, 3, p. 647.

Two hypotheses can be put forward to account for this change in the third component; first, that certain substances are absorbed from the third component by the corpuscles during such exposure; second, that the corpuscles give off into the third component substances that influence its action. Both hypotheses were tested.

In order to determine whether or not substances are absorbed from the third component, washed corpuscles were exposed to a third component having the maximum hemolysis-increasing power; they were then washed free from the serum and their susceptibility to hemolysis compared with that of unexposed corpuscles. No change was found in susceptibility after such exposure. The third component, therefore, is either not absorbed by corpuscles, or, if absorbed, is held in such a loose chemical union that it is easily removed by successive washings. Absorption is experimentally undemonstrable.

To determine whether or not substances are given off by corpuscles into the third component, washed corpuscles were exposed to physiological saline, under conditions identical with those of the absorption experiments above, the salt solution simply taking the place of the heated serum. The physiological saline was then freed from corpuscles by centrifugation, and its effect on hemolysis tested.

In all cases, it was found that the exposed salt solution was strongly antilytic. Corpuscles, therefore, give off an hemolysis-inhibiting substance into the third component. Whether or not, this accounts for the total change in exposed third component has not as yet been determined.

Reverting now to the original problem of testing the physico-chemical law proposed for the absorption of hemolytic amboceptor, we see that when heated hemolytic serum is exposed to corpuscles, three phenomena occur: first, an as yet purely hypothetical absorption of amboceptor; second, a demonstrable change in the third component; and, third, a giving-off into the serum of antihemolytic corpuscle products.¹

No direct measurement, therefore, of the amboceptor-power of exposed serum will give any idea whatever of the amount of amboceptor remaining in it. Experimental proof or disproof of the proposed physico-chemical law is therefore at present impossible.

¹ The second and third changes may, of course, be identical.

Similarly, the presence of an active third component, which differs in different sera prepared under identical conditions, prevents the experimental verification of the physico-chemical law proposed for the interaction of hemolytic complement and amboceptor. *The physical chemistry of hemolytic serum is, therefore, beyond the present reach of experimental science.*